IS 548-2-22 (1993): Methods of sampling and test for oils and fats, Part 2: Purity test, Section 22: Detection of tricresyl phosphate in edible oil [FAD 13: Oils and Oilseeds]

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(Reaffirmed 1999)
2009

भारतीय मानक

तेल और वसा के लिये नमूने लेने तथा परीक्षण की पद्धति

भाग 2 शुद्धता परीक्षण

अनुभाग 22 खाद्य तेलों में ट्राइक्रिसाइल फास्फेट का संसूचन

Indian Standard

METHODS OF SAMPLING AND TEST FOR OILS AND FATS

PART 2 PURITY TESTS

Section 22 Test for Detection of Tricresyl Phosphate in Edible Oil

UDC 665·3 : 543·869

BUREAU OF INDIAN STANDARDS
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May 1993

Price Group 1

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Oils and Oilseed Sectional Committee had been approved by the Food and Agriculture Division Council.

Indian Standard Methods of Sampling and Tests for Oils and Fats (IS 548) was first published in 1954 and subsequently revised in 1964 as Part 1 and it covered methods of sampling and physical, chemical and qualitative tests.

In view of periodical review of qualitative tests for detection of adulteration in oils and fats, the concerned Sectional Committee decided to cover such tests in Part 2 of this standard and IS 548 (Part 2) : 1976 Method of sampling and tests for oils and fats: Part 2 Purity tests was accordingly published.

The Sectional Committee felt that additional purity tests should be covered in the form of separate test methods and should not be added as amendments to the standard (that is, Part 2) since it would create confusion. The tests covered under various sections of IS 548 (Part 2) : 1976 are as follows:

Section 6 Test for the presence of sesame oil (modified Baudouin test);

Section 7 Test for the presence of cottonseed oil (Halphen test);

Section 8 Test for the presence of linseed oil (Hexabromide test);

Section 9 Test for the presence of *Karanja* (*Pungam*) oil in other oils;

Section 10 Test for the presence of argemone oil by paper chromatographic method;

Section 11 Test for the presence of hydrocyanic acid;

Section 12 Test for the presence of mineral oil;

Section 13 Test for the presence of ground nut oil [Bellier turbidity temperature test (acetic acid method)]

Section 14 Test for the presence of *Kusum* oil and other oils containing cyanogenic compounds;

Section 15 Test for the presence of castor oil:

Section 16 Test for the presence of *Neem* oil;

Section 17 Test for the presence of other oils in castor oil;

Section 18 Test for the presence of animal fat in vegetable oil (phytosterol acetate melting point test);

Section 19 Test for the presence of oil soluble colours;

Section 20 Test for detection of Taramira oil in mustard rapeseed oil; and

Section 21 Test for detection of animal fat in vegetable oils and fats and *vice-versa* by GLC.

Tricresyl phosphate, also known as tritolyl phosphate is used as a plasticizer. This is quite toxic and cases of poisoning from this phosphoric ester causing paralysis have been reported from time to time. A plastic container which has held tricresyl phosphate and which has been improperly rinsed (due to its insolubility in water) is sufficient to contaminate a vegetable oil subsequently put into it.

Section 22 prescribes a simple and rapid method for detection of tricresyl phosphate in edible oils.

In reporting the result of a test or analysis made in accordance with this standard, the final value, observed or calculated, is to be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

METHODS OF SAMPLING AND TEST FOR OILS AND FATS

PART 2 PURITY TESTS

Section 22 Test for Detection of Tricresyl Phosphate in Edible Oil

1. SCOPE

1.1 This standard (Part 2/Section 22) describes a thin layer chromatographic method for detection of tricresyl phosphate in edible oils.

2 REFERENCE

The following Indian Standard is a necessary adjuncts to this standard.

IS No.	*Title*
323 : 1959	Rectified spirit (*revised*)

3 PRINCIPLE

3.1 The method is based on the alkaline hydrolysis of the oil sample followed by detection of cresols by diazonium reagent.

4 APPARATUS

4.1 Conical Flask

250-ml capacity fitted with air condenser.

4.2 Glass Plates

10 x 20 cm.

4.3 Glass Tank with Lid

For developing 10 x 20 cm plates.

4.4 Spreader

Fitted with screw gauge for adjustment of slurry thickness.

4.5 Sprayer

4.6 Air Oven

With temperature controlling system

4.7 Desiccator

For preserving the TLC plate

4.8 Water Bath or Hot Plate

Rheostat control

4.9 Capillary Tube or Micropipette

4.10 Pipette

5-ml and 50-ml capacity.

5 REAGENTS

5.1 Alcoholic Potassium Hydroxide Solution

Dissolve 70 to 80 g of potassium hydroxide in an equal quantity of distilled water and add 2 litres of aldehyde-free alcohol or rectified spirit (*see* IS 323 : 1959). Allow to stand overnight, decant the clear liquid and keep in a bottle closed tightly with a cork or rubber stopper.

5.2 Alcoholic Potassium Hydroxide Solution

1.5 N. Add 8.5 g of potassium hydroxide in 100 ml of aldehyde-free alcohol or rectified spirit.

5.3 Diazomium Reagent

Dissolve 0.8 g *p*-nitroaniline (AR grade) in 250 ml luke warm distilled water. Add 20 ml of 20 percent hydrochloric acid to the lukewarm mixture and mix properly to dissolve the *p*-nitroaniline. Decant to remove any residual slick which remains. Cool and then add 50 percent sodium nitrite ($NaNo_2$) solution until the reagent is entirely colourless. Keep the reagent in a reagent bottle and store in refrigerator.

5.4 Iso-octane

AR grade

5.5 Ethylacetate

AR grade

5.6 Tricresly Phosphate Standard

Prepare 0.5 percent solution of tricresyl phosphate (AR grade) in pure rapeseed oil.

6 PROCEDURE

6.1 Saponification

6.1.1 Weigh accurately 5 g of the well-mixed sample into a conical flask. Add 50 ml of alcoholic potassium hydroxide solution.

6.1.2 Take 15 ml of standard sample solution (**5.6**) in another conical flask and add 50 ml of alcoholic potassium hydroxide solution.

6.1.3 Fit both the flasks with air condenser and boil gently but staedily on water bath or hot plate for one hour or until the saponification is complete.

6.2 Isolation by Thin Layer Chromatography

6.2.1 *Preparation of the Chromatoplates*

Wash the glass plates thoroughly with a detergent solution and water, and dry with acetone in order to remove all traces of fatty matter. Dry the plates in the air, activate at 110°C in an air oven for one hour and preserve in a dessicator.

Place 60 g of silica gel G in a 500-ml conical flask. Add 120 ml of distilled water. Stopper and shake vigorously for one minute. Immediately pour the slurry into the spreader. Spread in a 300 mμ thick layer on the clean plates. Dry the plates for 30 minutes in an air oven and keep in a desiccator before use.

6.2.2 Prepare a mixture of isooctane ethyl acetate (90 : 10) which shall be used for development of the spots in the tank under saturated condition with solvent vapour.

6.2.3 Spot 10-20 µl of the saponified sample as well as standard (**6.1**) on TLC plates (**6.2.1**) with capillary tube or micropipette and place the plates properly in the developing tank (**6.2.2**) and allow the solvent to run at least up to 10 cm (15 minutes) on the plates. After development, dry the plates in air spray with 1.5 N alcoholic potassium hydroxide and then heat at 60°C for 15 minutes in the air oven. Now spray the plate with diazomium reagent (*see* **5.3**).

6.2.4 Appearance of red spots at the same Rf of standard sample confirms the presence of tricresyl phosphate in the oil. The Rf is generally found at 0.42. The comparison of Rf should always be made with the reference sample

NOTES

1 The oil can be spotted directly without any previous treatment but it has been found that after hydrolysis, the coupling of cresols formed with diaotized *p*-nitraniline gives a better coloration.

2 Since red colour formation is most favourable in alkaline medium, the strength of alcoholic potash in preliminary spraying should preferably be at least 1.5 N, otherwise confusion may arise with light yellow spots.

3 The minimum detection limit of this method is 1.5 µg.

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Doc : No. FAD 44 (22)

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

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Printed at New India Printing Press, Khurja, India